

1. (Twice amended) An attenuated derivative of a pathogenic microorganism which [further] comprises:

- (a) a[n inactivating mutation in a] non-functional native chromosomal essential gene;
- (b) a recombinant complementing gene on an extrachromosomal vector [encoding a functional replacement for the essential enzyme], wherein the complementing gene can recombine to replace the [defective] non-functional native chromosomal essential gene; and
- (c) a desired gene on the extrachromosomal vector, wherein the desired gene is a recombinant gene encoding a desired gene product;

wherein said complementing gene of (b) is a functional replacement for said essential gene of (a), wherein the desired gene is stably maintained in a progeny population of the microorganism.

3. (Amended) The microorganism of claim 2, [further comprising an inactivating mutation] wherein the non-functional native chromosomal essential gene is a gene selected from the group consisting of a *pab* gene, a *pur* gene, an *aro* gene, *nadA*, *pncB*, *galE*, *pmi*, *fur*, *rpsL*, *ompR*, *htrA*, *hemA*, *cdt*, *cya*, *crp*, *dam*, *phoP*, *phoQ*, *rfc*, *poxA*, *galU*, *mviA*, *sodC*, *recA*, *ssrA*, *sirA*, *inv*, *hliA*, *rpoE*, *flgM*, *tonB*, and *slyA*.

7. (Amended) The microorganism of claim 6, wherein the [inactivating mutation is in an] non-functional native chromosomal essential gene is an *asd* gene [and] wherein said *asd* gene comprises an insertion or a deletion.

11. (Amended) The microorganism of claim 10, wherein the eukaryotic promoter is a CMV (cytomegalovirus) promoter.

12. (Amended) A recombinant vector comprising a recombinant complementing gene [encoding an essential enzyme], wherein the recombinant complementing gene lacks an RNA polymerase -35 recognition sequence and a promoter -10 sequence,

wherein the [vector can functionally replace the essential enzyme] recombinant complementing gene is a functional replacement for a non-functional native chromosomal essential gene when the vector is present in a microorganism having [an inactivating mutation in a native gene encoding the essential enzyme] a non-functional native chromosomal essential gene.

13. (Amended) The recombinant vector of claim 12, wherein the vector is a plasmid capable of expressing the [essential enzyme] recombinant complementing gene in a microorganism that is a member of the *Enterobacteriaceae*.

14. (Amended) The recombinant vector of claim 12, wherein the [essential] recombinant complementing gene encodes an enzyme that catalyzes a step in the biosynthesis of DAP (mesodiaminopimelic acid).

15. (Amended) The recombinant vector of claim 14, wherein the recombinant complementing gene is an *asd* gene.

16. (Amended) The recombinant vector of claim 12, further comprising a gene encoding a desired gene product.

#### Remarks

Claims 1, 3, 6-7, 11, and 12-16 have been amended. Claims 1-22 remain in the application. The following remarks address the rejections in the Office Action dated 6/20/01.

#### Rejections under 35 U.S.C. §112, second paragraph

Claims 1, 3, 6, 11-12 and 14 are rejected under 35 U.S.C. § 112, second paragraph as being indefinite.

The terms 'functional replacement' and 'functionally replace' in claims 1 and 12 are allegedly unclear, because it is not clear which functions of the essential enzyme will be replaced by the vector. Applicants first point out that claims have been amended to clear up any ambiguity associated with the respective terms. With respect to the term 'functional replacement' in claim 1, this term would be understood by the skilled artisan based on the discussion of essential genes in the specification. At page 14, line 30 through page 15, line 4, the term 'essential gene' is discussed. An 'essential gene' is defined as a gene that encodes a function that is required for cell viability. The discussion continues: 'Essential genes may be functional, that is they are providing the essential function. Essential genes may also be non-functional, for example by having mutations that render the translated protein non-functional, or by not being operably linked to a control element essential for transcription of the gene.' Claim 1 recites a 'non-functional' native chromosomal essential gene in (a). By looking to the definition of an essential gene in the specification, it is clear that a non-functional essential gene is one that does not provide the essential function that is required for cell viability. The term 'functional replacement' is used in claim 1 to